

BIOSYSTEMATIC STUDIES ON THE *CLOSTERIUM* *MONILIFERUM*/*EHRENBERGII* COMPLEX (CHLOROPHYTA, CONJUGATOPHYCEAE) IN WESTERN EUROPE. IV. DISTRIBUTIONAL ASPECTS.

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ABSTRACT - 58 Clones of *Closterium ehrenbergii* and 77 clones of *C. moniliferum* each isolated from 6 populations in Normandy (France) were investigated for isozyme banding patterns, sexual compatibility and morphological variability. With respect to all three aspects the *C. ehrenbergii* clones represented two groups, one fitting in with the sexuality heterothallic clones isolated earlier from England and one - more closely related to *C. moniliferum* - fitting in with the (so far) incompatible clones isolated earlier from Holland. All compatible *C. ehrenbergii* populations sampled from England and France originated from water bodies on sandy or clayey soil while the incompatible *C. ehrenbergii* populations sampled in Holland and France occupied peaty fen habitats. The possible significance of this phenomenon is discussed.

RÉSUMÉ - 58 Clones de *Closterium ehrenbergii* et 77 clones de *C. moniliferum* isolés chacun à partir de 6 populations en Normandie (France) ont été analysés par leurs profils électrophorétiques d'isoenzymes, leur compatibilité sexuelle et leur variabilité morphologique. D'après ces trois sortes d'investigations les clones de *C. ehrenbergii* présentaient deux groupes: l'un correspondant aux clones sexuellement hétérothalliques isolés précédemment d'Angleterre; l'autre - en rapport plus étroit avec *C. moniliferum* - correspondant aux clones (jusqu'ici) incompatibles isolés précédemment de Hollande. Toutes les populations compatibles de *C. ehrenbergii* provenant d'Angleterre et de France se trouvaient dans des points d'eau sur substrat sableux ou argileux, tandis que les populations incompatibles de *C. ehrenbergii* provenant de Hollande et de France occupaient des marais tourbeux. La signification de ce phénomène est discutée.

KEY WORDS : biosystematics, habitat differentiation, desmids, *Closterium moniliferum*, *Closterium ehrenbergii*.

INTRODUCTION

In previous papers we have demonstrated the occurrence of at least two biological entities within the typological species *Closterium ehrenbergii* as distributed in western Europe. *C. ehrenbergii* clones isolated from a number of sites in the southern part of England differed both in isozyme composition, sexual compatibility and morphology from *C. ehrenbergii* clones isolated from Holland (Coesel & Menken, 1988; Coesel, 1988a, b). Especially enzymatically and morphologically Dutch *C. ehrenbergii* clones resembled clones of the typological species *C. moniliferum* much more than the English ones did. As the Dutch habitats for *C. ehrenbergii* concerned peaty areas (fens, broads) while the English isolates originated from small streams and pools on solid soil, we wondered in how far the distribution of the two *C. ehrenbergii* types would be ecologically rather than geographically determined. We therefore collected *C. ehrenbergii* from Normandy, in the uppermost north-western part of France. This region, geographically intermediating S. England and The Netherlands, is rich in potential sites for *C. ehrenbergii*, both peaty and non-peaty in nature. The results of investigation into this Norman material of *C. ehrenbergii* (and *C. moniliferum* for comparison) are dealt with in this paper.

MATERIAL AND METHODS

The north-western part of Normandy was visited in early spring 1985 and 1986. *C. ehrenbergii* and *C. moniliferum* were sampled from a number of marshy sites along the rivulets Canche and l'Authie as well as from some shallow pools at the coast. Details about sampling sites and number of clones isolated are given in Table I. For methods concerning analysis of isozymes see Coesel & Menken (1988), for mating procedures Coesel (1988a) and for morphological characterization Coesel (1988b).

RESULTS

Sexual compatibility

All clones were tested for homothallism. In testing heterothallism, numerous intra- and interpopulation combinations of clones were tried out. Attempts to induce sexual reproduction failed completely with *C. moniliferum* clones and also to the *C. ehrenbergii* clones originating from the inland sites. However, all 40 clones from the four coastal *C. ehrenbergii* populations appeared compatible in certain mutual combinations. In addition, all these clones could be mated with a pair of *C. ehrenbergii* tester strains from England (Table II). Although the percentage of cells fusing into zygosporangia varied considerably with any specific combination of plus and minus strain (as it does in intrapopulation combinations), we consider these 40 heterothallic clones from the Norman coast to belong to one and the same mating group as our English *C. ehrenbergii* clones. Success of mating was independent of differences in size between partner cells (Fig. 1-4).

Popul.	Species	Geographic site	Locality	Habitat	No. of isolates
A	<i>C. ehrenb.</i>	coast	Trepied	dune pool	4
■	" "	"	Haringzelles	bunker canal	15
C	" "	"	Cap Gris-Nez	bomb crater	12
D	" "	"	"	"	9
E	" "	inland	Dominois	fen hollows	10
F	" "	"	Marenla	"	■
G	<i>C. monilif.</i>	"	"	"	5
H	" "	"	Dominois	"	■
I	" "	"	Roussent	"	6
J	" "	"	Argoulas	"	11
K	" "	coast	Ambleteuse	dune pool	6
L	" "	"	"	meadow pool	41

Table I - Origin of the Norman populations of *Closterium ehrenbergii* and *C. moniliferum*.

Isozyme patterns

When computing phenotypic identity values between all pairwise combinations of Norman populations of *C. ehrenbergii* and *C. moniliferum* a marked difference appears between the compatible *C. ehrenbergii* populations from the coastal region and the incompatible ones from the inland (Table III). Just like the incompatible *C. ehrenbergii* populations from Holland in the incompatible Norman *C. ehrenbergii* populations are much more closely related to *C. moniliferum* than to compatible *C. ehrenbergii* populations. The relationship between compatible and incompatible *C. ehrenbergii* populations from France, England and Holland is shown in Table IV. The compatible *C. ehrenbergii* popu-

Popul.	No. of clones	Type "plus"	Type "minus"
A	4	3	1
■	15	7	■
C	12	7	5
D	9	4	5

Table II - Distribution of plus and minus mating type in the coastal populations of *Closterium ehrenbergii* from Normandy. Labelling of mating type according to compatibility with the English *C. ehrenbergii* tester strains 799 (+) and 800 (-).



Fig. 1-4 - Sexual reproduction between English and French clones of *C. ehrenbergii*. Figs. 1-2: Strain n° 742 (mating type minus clone from English population B) combined with strain n° 1985 (mating type plus clone from French population B). Strain n° 742 left, n° 1985 right in picture. Figs. 3-4: Strain n° 800 (minus clone from English population C) combined with strain n° 1985 (plus clone from French population B). Strain n° 800 left, n° 1985 right in picture. Scale bar: 200 μ m.

	<i>C. ehrenb.</i> coast	<i>C. ehrenb.</i> inland	<i>C. monilif.</i>
<i>C. ehrenb.</i> — coast	0.94		
<i>C. ehrenb.</i> — inland	0.18	0.99	
<i>C. monilif.</i>	0.19	0.80	0.87

Table III - Mean phenotypic isozyme identity within and between groups of Norman populations of *Closterium moniliferum*, *C. ehrenbergii* (coast) and *C. ehrenbergii* (inland), averaged over five enzymes tested (CAT, ME, SDH, ACPH and IDH).

	<i>C. ehrenb.</i> England (compatible)	<i>C. ehrenb.</i> Holland (incompatible)
<i>C. ehrenb.</i> — France/compatible	0.81	0.34
<i>C. ehrenb.</i> — France/incompatible	0.01	0.62

Table IV - Mean phenotypic isozyme identity between both compatible and incompatible groups of populations of *Closterium ehrenbergii* from France (Normandy), Holland, and England (averaged over the five enzymes tested).

lations from France are closely related to the English ones indeed, just as the incompatible populations from France with respect to the Dutch ones.

Morphological variability

Mean values plus standard deviations and extremes for cell length, cell width and degree of curvature per population are presented in Figs 5 and 6. Although there is some overlap especially in cell size between compatible and incompatible Norman populations of *C. ehrenbergii* (Fig. 5) these data are in rough agreement with those from England and Holland. That is, the incompatible *C. ehrenbergii* populations are morphologically more or less intermediate between compatible *C. ehrenbergii* populations and *C. moniliferum* populations (Coesel, 1988b). For an outline of these morphological characteristics, see Table V as for the Norman populations and Table VI as for the total of sampled French, English and Dutch populations.

DISCUSSION

The above discussed results indicate the occurrence in Normandy of two types of *C. ehrenbergii*. One of these is sexual compatible, the other one is not, at least it could not be induced to sexual activity under the conditions and in the clonal

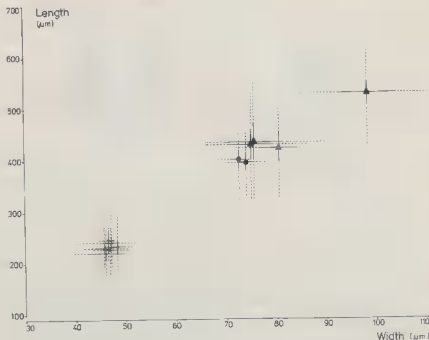


Fig. 5 - Variability in cell length and width in Norman populations of *C. moniliferum* (mean values indicated as dots), *C. ehrenbergii* from inland sites (mean values indicated as circles) and *C. ehrenbergii* from coastal sites (mean values indicated as triangles). Solid lines indicate standard deviations, dotted lines extreme values encountered in any of the clones belonging to a given population.

combinations tried out. Just as in the previously studied Dutch-English material, the incompatible *C. ehrenbergii* populations resembled *C. moniliferum* much more than the compatible ones do, especially in isozymes and, to a lesser extent, morphologically. Moreover, the Norman compatible clones of *C. ehrenbergii* appeared to constitute part of the same mating group as the English compatible clones. There was also a high isozyme identity between these two groups of clones as well as between incompatible *C. ehrenbergii* clones from Normandy and incompatible clones from Holland. All these data reveal that the two biological entities within *C. ehrenbergii* that were encountered earlier in England and Holland respectively, are both present in Normandy.

The two types of *C. ehrenbergii* were never found together at one and the same site. Though at first sight the distribution of these types seems to be determined geographically, most probably ecological parameters play a decisive role. The shortest geographical distance between localities with compatible and those

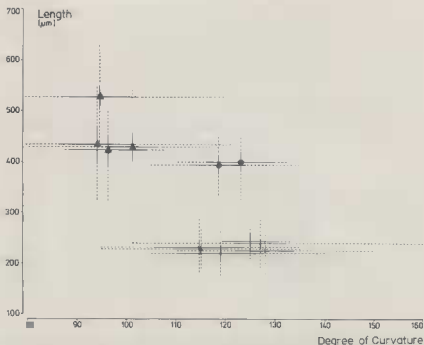


Fig. 6 - Variability in cell and curvature in Norman populations of *C. moniliferum* (dots), *C. ehrenbergii* from inland sites (circles) and *C. ehrenbergii* from coastal sites (triangles). Symbols as in Figs. 5.

with incompatible populations of *C. ehrenbergii* as traced in Normandy, is not more than some fifty kilometers. It is very unlikely that migrating waterbirds acting as potential vectors in dispersal of desmids (e.g. Proctor, 1966) do not bridge those sites. However, when the various habitats from which *C. ehrenbergii* was isolated are compared, all compatible populations of this species sampled in England and France appear to occur in shallow waterbodies on sandy or clayey soil that regularly dry out in dry summers. On the contrary, all incompatible populations sampled in Holland and France are from peaty sites that seldom or never dry up. One may imagine that water bodies regularly falling dry form an unstable environment where sexual reproduction may be of substantial advantage. Not only in terms of survival of the resistant zygospores in the dry habitat but also in maintaining a high genetic diversity with a big potential for selection (e.g. Coleman, 1983). It is striking that most of the compatible *C. ehrenbergii* strains studied in Asia by Ichimura and Kasai (e.g. Ichimura, 1981; Ichimura & Kasai, 1982) originate from paddy fields that are well known for their changeable water level. Also Blackburn & Tyler (1987) report frequent conjugation in a wide variety of desmid genera, especially from unstable (tropical) habitats.

	Cell length (µm)			Width (µm)			Curvature (°)		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
<i>C. ehrenb.</i> — compatible	423-528	453	50.1	75-98	82	11.0	94-101	96	3.2
<i>C. ehrenb.</i> — incompatible	394-400	397	4.2	73-74	73	1.0	119-123	121	3.2
<i>C. monilif.</i>	220-246	233	9.3	46-48	47	0.9	115-128	122	5.9

Table V - Morphological characteristics, based on mean population values, clones of *Closterium moniliferum* and *C. ehrenbergii* (both compatible and incompatible) from Normandy. (Mean population values based on 25 randomly selected cells per clone).

	Cell length (µm)			Width (µm)			Curvature (°)		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
<i>C. ehrenb.</i> — compatible	423-555	491	54.4	75-102	87	9.8	94-107	100	5.1
<i>C. ehrenb.</i> — incompatible	366-400	382	11.9	65-74	71	3.5	110-123	115	4.7
<i>C. moniliferum</i>	207-246	229	11.1	36-48	44	3.4	106-128	117	7.2

Table VI - Morphological characteristics (based on mean population values) for the total of clones of *Closterium moniliferum* and *C. ehrenbergii* (both compatible and incompatible) sampled from England, Holland and France. (Mean population values based on 25 randomly selected cells per clone).

In summary, we may conclude that the nomenclotypes *C. ehrenbergii* as occurring in western Europe can be split up into (at least) two biological entities, one of them much closer related to *C. moniliferum* than the other one. Both entities are mutually completely sexually isolated and, in addition, occupy different habitats. It would be very interesting to see how these both *C. ehrenbergii* types fit into the scale of mating groups distinguished by Ichimura (see e.g. Ichimura, 1985) for other parts of the world.

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